

CORRELATION OF SALMONELLA SPP. IN PIGS AT SLAUGHTER AS DETERMINED BY BACTERIAL CULTURE AND SALMONELLA ELISA

BURKHART, K.^a, OPPEDAHN, A.^a, ROOF, M.^a, KOLB, J.^b, NIELSEN, B.^c

The purpose of this study was to determine the prevalence of Salmonella spp. in pigs at slaughter by bacterial culture, and serology using the Danish Mix-ELISA. Fecal samples and mesenteric lymph nodes were collected from 20 farms (30-35 samples per farm) and cultured for Salmonella on XLD agar following a pre-enrichment in buffered peptone water at 37° C for 24 hours and enrichment in Rappaport-Vassiliadis broth at 42° C for 24 hours. Eighteen of 20 farms yielded at least one positive sample by culture with farm sample prevalence ranging from 3.3% to 96.6%. There were 16 different serotypes of Salmonella isolated. Individual farms had from 0 - 6 different serotypes detected by bacteriologic examination. The Danish Mix-ELISA, developed by Nielsen et al and currently used in the Danish slaughter plants, was used to test sera collected at slaughter. Using the Mix-ELISA, 16/20 farms were positive with farm sample prevalence ranging from 28.6% to 100%. In comparing the farms, there was a direct correlation between lymph node culture and Mix-ELISA detection levels. These data suggest that the Mix-ELISA may be a valuable herd screening tool for the evaluation of Salmonella levels in U.S. swine herds. Also, these data suggest that vaccination of pigs with SC-54 significantly reduces the prevalence of Salmonella when measured by serology, mesenteric lymph node culture, and fecal culture.

INTRODUCTION

Reduction of Salmonella in pork products has become an increasingly important issue in regards to food safety. Infected herds constitute a public health risk as a potential source of contamination of carcasses at slaughter. Bacterial culture of fecal samples has been the most widely used method of detecting Salmonella infections among herds. In the early stages of infection, bacteriologic methods are generally sufficient, but in later stages, pigs may become intermittent shedders and may not be detected by culture. The Danish Mix-ELISA, developed by Dr. Bent Nielsen, is currently being used in Denmark as a herd monitoring tool to detect herds with high levels of Salmonella infections prior to slaughter. The Mix-ELISA utilizes LPS antigen from both S. typhimurium and S. choleraesuis. The goal of the study was to determine if there is a correlation between bacteriologic culture and the serologic status of swine herds prior to slaughter.

MATERIALS AND METHODS

Experimental Design

1. Pigs sampled were from 20 commercial farms with > 8000 sows per farm
2. Each farm was a multisight facility, farrow to finish, with 30 pigs per finisher
3. 30 pigs were sampled from each farm, collected mesenteric lymph node, feces, and sera from each pig
4. Twelve farms were vaccinated, eight were not vaccinated

^a NOBL Laboratories, Inc., Ames, Iowa, USA

^b NOBL Laboratories, Inc., Sioux Center, Iowa, USA

^c Danske Slagterier, Copenhagen, Denmark

Bacterial Culture Procedure

1. Salmonella culture was done on mesenteric lymph nodes and fecal samples.
2. Tissue samples were homogenized and approximately one gram of tissue and one gram of feces was put into 10 ml of Buffered Peptone Water , and the tubes were incubated at 37° C for 24 hours.
3. 100 ul of the buffered peptone water was transferred to 10 ml of RV Broth (Rappaport-Vassiliadis), and the tubes were incubated at 42° C for 24 hours.
4. Each sample was streaked onto XLD agar plates and incubated at 37° C for 24 hours.
5. Suspected colonies were inoculated into Kligler, Urea, and SIM agar tubes and incubated at 37° C for 24 hours.
6. Isolates confirmed to be Salmonella were submitted to the National Veterinary Services Laboratory for serotyping.

Danish Mix-ELISA Procedure

1. Immulon 2 flat bottom plates were coated with S. typhimurium and S. choleraesuis LPS antigen diluted in WFI water to 240 ng and 300 ng/well respectively. 100 ul was added to each well.
2. Plates were covered and incubated at room temperature overnight.
3. Coating buffer was discarded and plates were blocked with 100 ul / well with PBS-T-BSA for 30 minutes.
4. Liquid was discarded and plates were washed once with PBS-T
5. Serum was diluted 1:400 in PBS-T-BSA and 100 ul was added to each well. Plates were incubated at room temperature for 90 minutes.
6. Plates were washed three times with PBS-T.
7. The conjugate, horse radish peroxidase labeled rabbit antiserum pig Ig, was diluted 1:1000 in PBS-T-BSA. 100 ul of conjugate was added to each well, and plates were incubated at room temperature for one hour.
8. Plates were washed three times with PBS-T.
9. 100 ul of OPD solution (Citrate Buffer, pH 5) was added to each well, and plates were incubated at room temperature in the dark for 10 minutes
10. 100 ul of 0.5 M Sulfuric acid was added to each well to stop the process.
11. Plates were read on a Dynatech plate reader at 490 nm with a background correction of 650 nm.

Results were calibrated using a reference sera and a standard curve was used for positive samples. An OD % of 40 was the cut-off.

RESULTS

Culture Results:

18 / 20 (90%) of farms positive by culture

Range from 3.3% to 86.2% positive from mesenteric lymph nodes

Range from 3.3% to 96.6% positive from fecal samples

16 serotypes were isolated

1-6 different serotypes cultured per farm

11 different serotypes were cultured from the MLN and 12 serotypes were cultured from the fecals.

7 different seotypes were common to both, MLN had 4 unique serotypes and fecals had 5 unique serotypes

Salmonella detection was significantly lower among the vaccinated farms in serology, lymph node culture, and fecal culture.

Serology Results:

13 / 17 (76.5%) of farms positive by Mix-ELISA

Range from 2.9% to 91.4% positive

The correlation co-efficient between the MLN and Mix-ELISA was $R = .67$

DISCUSSION

The results of this investigation suggest that Salmonella infection at slaughter is possible even under today's improved management systems. Use of feces and mesenteric lymph nodes as monitoring samples is feasible, although the number and serotype isolated may be different among samples. Evaluation of the Danish Mix-ELISA also suggested a good correlation ($R=.67$) between mesenteric lymph nodes and serum ELISA. This indicates that either or both tools may be important in Salmonella monitoring as a part of a reduction program.

With a majority of farms testing positive for Salmonella, the need for a test system that is faster and more economical than culture is required. The potential advantages of a serological testing include reduced cost, quicker results, and identification of previously exposed pigs which are not actively infected or shedding salmonellae. One herd (CN) had a high recovery rate of Salmonella from both the lymph nodes and feces and yet was seronegative. It is speculated that this may be a case of acute exposure and seroconversion had not yet occurred. As samples were collected at slaughter, no additional samples were collected to evaluate this hypothesis.

It was also noted that herds that were vaccinated with an avirulent live Salmonella choleraesuis vaccine (SC-54) had significantly ($P<0.05$) less Salmonella recovered at slaughter than did non-vaccinated herds. This suggests that vaccination may not only be an important tool to control clinical disease, but also aid in the reduction of Salmonella levels at slaughter and ultimately improved food safety.

In conclusion, culture of mesenteric lymph nodes and serology using the Danish Mix-ELISA should be considered in Salmonella monitoring programs. Use of an avirulent live vaccine along with other management tools may reduce overall herd Salmonella levels and ultimately improve food safety.